The Amendments

In the Specification:

Amend the paragraph starting at page 12, line 23:

According to the present invention, all or part of the molecular components of the raw samples are solubilized in a suitable lysis buffer comprising e.g. solvents. Such solvents may for example be aqueous solutions of chaotropic agents such as e.g. urea, GuaSCN, Formamid, of detergents such as anionic detergents (e.g. SDS, N-lauryl sarcosine, sodium deoxycholate, alkyl-aryl sulphonates, long chain (fatty) alcohol sulphates, olefine sulphates and sulphonates, alpha olefine sulphates and sulphonates, sulphated monoglycerides, sulphated ethers, sulphosuccinates, alkane sulphonates, phosphate esters, alkyl isethionates, sucrose esters), cationic detergents (e.g. cetyl trimethylammonium chloride), non-ionic detergents (e.g. ~~Tween 20,~~ TWEEN®-20, polyethylene glycol sorbitan monolaurate; ~~Nonidet~~ nonidet P-40, ~~Triton X-100,~~ TRITON® X-100, t-octylphenoxypolyethoxyethanol; NP-40, ~~Igepal CA-630,~~ IGEPAL® CA 630, nonidet P 40; N-Octyl-Glucosid) or amphoteric detergents (e.g CHAPS, 3-Dodecyl-dimethylammonio-propane-1-sulfonate, Lauryldimethylamine oxide) and/or of alkali hydroxides such as e.g. NaOH or KOH. The solvent is designed, so that cells, cell debris, nucleic acids, polypeptides, lipids and other biomolecules potentially present in the raw sample are dissolved. The solution for dissolving the raw samples according to the present invention may furthermore comprise one or more agents that prevent the degradation of components within the raw samples. Such components may for example comprise enzyme inhibitors such as proteinase inhibitors, RNAse inhibitors, DNAse inhibitors etc. In one embodiment of the present invention the sample is lysed directly in the form it is obtainable from the test-individuals. In another embodiment of the present invention the sample may be further purified before being lysed. Such purification procedures may for example comprise washing away of contaminants such as mucus or the like, separation or concentration of cellular components, preserving and transporting of the cells. Thus the cellular components of the raw samples are included in a single sample solution.

Amend the paragraph starting at page 17, line 25:

Marker molecules for use according to the present invention may comprise one or more

markers chosen from p13.5, p14, p15, p16 (also referred to $p16^{INK4a}$, SEQ ID NO: 13), p19, p21, p27, p53, pRb, p14ARF (SEQ ID NO: 14), cyclin A, cyclin B, cyclin E, MDM-2, MCM2, MCM5, MCM6, CDC2, CDC6, Id1, osteopontine, GRP, renal dipeptidase, her2/neu, TGFßII receptor, HPV associated markers e.g. derived from HPV genes L1, L2, E1, E2, E4, E5, E6 or E7, etc. A selection of markers useful in one embodiment of the present invention for the detection of medically relevant conditions is shown below in Table 1.

Amend the paragraph starting at page 20, line 13:

In certain embodiments normalization may also comprise proving the adequacy of the test, wherein as the case may be inadequate test results may be discarded or classified as invalid. ~~Therefore ,normalization~~ Therefore, normalization as used in the context of the present invention may comprise qualitative or semi-quantitative methods for normalization. In certain embodiments, semi-quantitative normalization may comprise determining a threshold value for a normalization marker. In one embodiment, semi-quantitative normalization may be applied e.g. as follows: the level determined for the relevant marker may be regarded as a valid test result only if the level of the normalization marker exceeds a defined threshold value; in case the threshold value is not reached the test result for the relevant marker is regarded as invalid; diagnosis may not be assessed on the basis of the test. In other embodiments a threshold may be set that may not be exceeded. In certain embodiments, qualitative normalization may be performed with respect to the presence or absence of a normalization marker. In those cases, e.g. the value determined for the relevant marker is compared to the presence or absence of a normalization marker. As predefined, the value is valid only in case the normalization parameter (presence or absence of a detectable level of the normalization marker) is met.

Amend Table 1 at page 21:

marker for	cell type	antigen	Antibody	supplier	Literature
cell type	epithelial cells	human epithelial cell surface glycoprotein	HEA125 IgG1 (W, IHC, ICC, IF)	Research Diagnostics Inc.	Kommoss et al., Hum Pathol. 2000 Sep;31(9):1055-61
		Human epithelial proliferation 40 kD protein (from LoVo)	AUA-1 IgG1 (Elisa)	Research Diagnostics Inc.	Gottschalk et al, Pathol Res Pract. 1992 Feb;188(1-2):182-90
		Human epithelial antigen (34+39 kD)	Ber-EP4, IgG1 (IHC, Elisa)	Dako	Latza U et al., J Clin Pathol. 1990 Mar;43(3):213-9
		Human epithelial proliferating antigen (40 kD)	AUA-1 (Elisa, W, IHC)	Research Diagnostics Inc.	Epenetos, A et al., Lancet. 1982 Nov 6;2(8306):1004-6
	endocervix columnar cells	Cytokeratin 18 (45 kD) SEQ ID NO: 9	RGE 53, IgG1 (W, IHC, IF)	Research Diagnostics Inc.	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68
		Cytokeratin 18 (45 kD	RCK 106 (W, IHF, IHC)	Research Diagnostics Inc	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68
		Cytokeratin 8 (52.5 kD) SEQ ID NO: 8	CAM 5.2 (W, IHC)	BD PharMingen	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68
	Endocervical columnar cells	Mucin Antigens (Tn, STn, MUC1, MUC2	DF3	Centocor	Tashiro et al., Hum Pathol. 1994 Apr;25(4):364-72
	Endocervic Columnar cells	Concanavalin A receptor			Herckenrode et al., Br J Cancer. 1988 Mar;57(3):293-4 ; Koch et al., Br J Cancer. 1986 Jan;53(1):13-22
	Endocervix	GalNacTransferase Oligosaccharyltransferase			Chilton et l., Endocrinology. 1988 Sep;123(3):1237-44

Amend Table 2 starting at Page 28, line 1:

Alpha-1 Catenin (Swissprot Accession P35221; also known as Cadherin-associated protein Alpha E-Catenin) SEQ ID NO: 4	Squamous epithelia	High in normal cervical epithelium at cell-cell-boundaries Strong reduction in high grade SILS	de Boer et al., 1999, Am J of Pathol, 155:505-515
Alpha-2 Catenin (Swissprot Accession P26232; also known as Alpha-Catenin related protein Alpha N-Catenin) SEQ ID NO: 5			
beta-Catenin (Swissprot Accession P35222 also known as PRO2286) SEQ ID NO: 6	Squamous epithelia	High in normal cervical epithelium at cell-cell-boundaries Strong reduction in high grade SILS	de Boer et al., 1999, Am J of Pathol, 155:505-515
Desmoplakin (Swissprot Accession P15924; also known as DP 250/210 kDa paraneoplastic pemphigus antigen)	stratified epithelia, simple epithelia, including glands, urothelium, thymic reticular epithelium, hepatocytes, intercalated disks of myocardium and arachnoid cells of meninges suprabasal layers of cervix (Superficial cells largely negative)	↓ in HSIL area	de Boer et al., 1999, Am J of Pathol, 155:505-515

Amend Table 3 at Page 29:

Table 3

Marker	**Histological testing**	**Cytological testing**	**Western Blot Analysis (clinical samples were freshly lysed with MTM buffer)**
E-Cadherin (Swissprot Accession P12830; also known as	**Squamous epithelia, (Parabasal,**	Parabasal, intermediate cells, no columnar cells	Only weak signal for HT-29. All clinical samples are negative

Uvomorulin, Cadherin-1, CAM 120/80; e.g.epitope: C-Terminus; AA735-883) SEQ ID NO: 3	**intermediate cells)** no columnar epithelia		
p120 Swissprot Accession O60716; p120 catenin, p120(ctn), Cadherin-associated Src substrate, CAS, p120(cas); e.g.epitope: C-Terminus; AA790-911) SEQ ID NO: 12	Squamous epithelia, (Parabasal, intermediate cells) also very strong in columnar epithelia	Very strong staining of parabasal, intermediate cells strong columnar cells	Only negative control (lymphocytes) and positive control (C4.1) positive
gamma-Catenin Swissprot Accession Q86W21; also known as Plakoglobin; e.g. epitope: C-Terminus; AA553-738) SEQ ID NO: 1	Squamous epithelia, (Parabasal, intermediate cells) no columnar epithelia, total epithelium is stained indysplasia	Very strong staining of parabasal, intermediate cells no columnar cells	Double bands (82/95 kD) in 60 % of samples (9/15); after acetone precipitation of 150 µl of samples: 87% (13/15) positive
Ep-Cam (Tumor-associated calcium signal transducer 1, Swissprot Accession P16422; also known as Major gastrointestinal tumor-associated protein, GA733-2, Epithelial cell surface antigen, Epithelial glycoproteins, EGP, Adenocarcinoma-associated antigen KSA KS 1/4 antigen Cell surface glycoprotein Trop-1) SEQ ID NO: 2	strong columnar epithelia, at very high concentrations rather unspecific (cytoplasmic) staining of squamous epithelia, (Parabasal, Intermediate Cells)	strong columnar cells, at very high concentrations rather unspecific (cytoplasmic) staining of squamous epithelia, (Parabasal, Intermediate Cells)	
Involucrin (Swissprot Accession P07476) SEQ ID NO: 7	Strong staining of squamous epithelia, (Parabasal, Intermediate Cells) and columnar epithelia; unspecific staining of stromal cells;	All cells and structures positive	

Amend the paragraph starting at page 32, line 7:

The test kit may optionally include a lysis buffer for solublization of the raw sample. Generally the lysis buffer may be any suitable solvent known to those of skill in the art. The lysis buffer for use in the kit may for example be aqueous solutions of chaotropic agents such as e.g. urea, GuaSCN, Formamid, of detergents such as anionic detergents (e.g. SDS, N-lauryl sarcosine, sodium deoxycholate, alkyl-aryl sulphonates, long chain (fatty) alcohol sulphates, olefine sulphates and sulphonates, alpha olefine sulphates and sulphonates, sulphated monoglycerides, sulphated ethers, sulphosuccinates, alkane sulphonates, phosphate esters, alkyl isethionates, sucrose esters), cationic detergents (e.g. cetyl trimethylammonium chloride), non-ionic detergents (e.g. ~~Tween 20~~ TWEEN®-20, ~~Nonidet~~ nonidet P-40, ~~Triton X-100~~ TRITON® X-100, NP-40, ~~Igepal CA-630~~ IGEPAL® CA 630, N-Octyl-Glucosid) or amphoteric detergents (e.g CHAPS, 3-Dodecyl-dimethylammonio-propane-1-sulfonate, Lauryldimethylamine oxide) and/or of alkali hydroxides such as e.g. NaOH or KOH.

Amend Table 4 at page 33:

Lysis buffer	**solubilization of p16INK4a in Western blot**	**compatibility with Elisa**
Detergents:		
0.1-1% SDS	+	+/-
0.2-3% SDS	+	< 0.5 %
0.2-3% DOC	++	+/-
0.1-1% n-Octylglycoside	+	yes
0.1-3% ~~Triton x-100%~~ TRITON® X-100	+	yes
0.1-1% Chaps	+	nd

Detergent-Mix:		
RIPA (1%NP40, 0.5%DOC, 0.1%SDS, PBS) 40-100%	++	yes
SOX (0.5% DOC, 0.5% n-Octylglycoside) 40-100%	+	yes
	++	yes
mtm lysis buffer (3% ~~Tritonx~~ TRITON® X-100, 0.4 % SDS, PBS)		

Amend the paragraph starting at page 37, lines 13:

In order to evaluate markers indicating the adequacy of cervical swabs, cervical sections (fixed in 4 % formaldehyde solution and paraffin-embedded) were stained with antibodies directed against Cytokeratin 18 (marker for endocervical columnar epithelia) and Cytokeratin 10/13 (SEQ ID NO: 10/11, marker for ectocervical squamous epithelia). Figure 1 shows specific staining of endocervical epithelia with anti-Cytokeratin 18 antibody and specific staining of ectocervical epithelia with anti-Cytokeratin 10/13 antibody. The experiment was performed as follows:

Amend the paragraph starting at page 40, line 9:

Western blot analysis was performed as follows: Patient samples were collected with a cervical brush and directly lysed in Laemmli Sample Buffer (2% SDS, 60mM Tris pH.6.8, 0.01%, 100 mM DTT) for 5 min at 95°C ($1x10^7$ cells/ml) with subsequent sonification (5x5sec pulses, maximum intensity). Lysates were centrifuged for 12 min at 16,600xg) in a microcentrifuge and supernatant was transferred into a new tube. Precast 4-20% linear gradient Acrylamide gels (Criterion System, Bio-Rad) were loaded with 10µl (10^5 cells) of whole cell extracts and proteins were separated at 25mA constant current for 45 min. Proteins were transferred from the gel to Hybond ECL Nitrocellulose membrane (Amersham) by standard tank blotting using the Bio Rad Criterion Blotter (15 min at constant 100 Volt and subsequently 45min at constant 50 Volt). Nitrocellulose-membrane was stained for 5 min in Ponceau S solution to assure protein transfer. Ponceau S solution was removed by 2x10 min washes in PBS.

For immunodetection, blots were blocked over night in blocking buffer (10% milk powder in PBS with 0.1% ~~Tween-20~~ TWEEN®-20). Primary antibodies were incubated at dilutions according to the manufacturer in blocking buffer for 1 h at RT with agitation (CK18: MAB 3236), 1:1000, CHEMICON; CK 10/13: DE-K13, 1:500, DAKO, $p16^{INK4a}$: D7D7, 1:140, MTM Laboratories). After 6 washes for 10 min with PBS/0.1% ~~Tween-20~~ TWEEN®-20, blots were incubated with rabbit anti mouse-HRP, (DAKO, diluted 1:5,000 in blocking buffer) for 1 h at RT. After 6 washes for 10 min with PBS/0.1% ~~Tween-20~~ TWEEN®-20, membranes were incubated for 5 min in substrate solution (Super Signal West Femto Maximum Substrate, Pierce), wrapped in a plastic envelope and exposed to an x-ray film for 1-5 min. Finally, x-ray films were developed, fixed, dried and documented with an imaging system (Bio-Rad). The same samples were used to perform ELISA analysis for $p16^{INK4a}$, CK 10/13, CK18. The detected signals and results were the similar to the Western blot analysis and the same conclusions were drawn.

Amend the paragraph starting at page 41, line 3:

The ELISA analysis was performed as follows: Flat bottom 96 well plates (MaxiSorb; Nunc) were coated with capture antibody ($p16^{INK4a}$: MTM-E6H4, 2µg/ml in PBS, MTM Laboratories; CK10: MS481P1ABX, 2µg/ml, dianova; CK18: K18.7, ~~2 g/ml~~ 2µg/ml, dianova; 50µl/well) over night at 4°C. Plates were washed 6x with PBS/0.1% ~~Tween-20~~ TWEEN®-20 and blocked with Superblock buffer (Pierce). Solubilized protein extract from cervical swabs were dissolved in incubation buffer (PBS, 3% ~~Superblock~~ SUPERBLOCK® (Chemical reagent), 0.1% ~~Tween20~~ TWEEN®-20), and added in triplicates to each well. After 1 h incubation at RT, plates were washed 6x with PBS/0.1% ~~Tween-20~~ TWEEN®-20 and incubated with biotinylated detection antibody ($p16^{INK4a}$: MTM-D7D7 (0.2 µg/ml, MTM Laboratories, CK10: MS481-BO, 200 µg/ml, dianova; CK18: MS142-BO, 200 µg/ml, dianova; in incubation buffer) for 1 h at RT. Following 6x washes withPBS/0.1% ~~Tween-20~~ TWEEN®-20 TMB, 50 µl of Streptavidin-coated Alkaline Phosphatase (1:1000 dilution; Dianova) was added for 30 min. Thereafter, plates were washed 6x with PBS/0.1% ~~Tween-20~~ TWEEN®-20 and 100 µl of p-nitrophenyl phosphate substrate (PnPP; dissolved in diethanol amine buffer) were added to each well. OD 405 nm (620 nm reference wavelength) was measured with an ELISA reader (~~Tecan~~ TECAN®) after 30 min,

1h and 2 hrs. The present example shows, that the sandwich ELISA format exhibits sensitivity, which is suitable for the use in the methods according to the present invention. For use in the method disclosed herein the sandwich ELISA format as described in this example may be applied to multiple marker molecules, such as markers for normalization/adequacy and markers characteristic for medically relevant conditions.

Amend the paragraph starting at page 44, line 1:

Coating solutions are removed from the ELISA plates and the plates are rinsed using an automated ELISA washer as follows:

- 7 x 250µl washing buffer (0.1% ~~Tween20~~ TWEEN®-20 (v/v) in PBS)

Amend the paragraph starting at page 48, line 21:

The ELISA plates are incubated at 25°C for exactly 15 min in the dark. Then the reaction is stopped by addition of 80 µl ~~2,5M H2SO4~~ 2.5M H_2SO_4.

Amend the paragraph starting at page 48, line 21:

Coating solutions are removed from the ELISA plates and the plates are rinsed using an automated ELISA washer as follows:

- 7 x 250µl washing buffer (0.1% ~~Tween20~~ TWEEN®-20 (v/v) in PBS)

After page 50, line 14, please start a new page and insert the attached paper copy of Sequence Listing pages 1-37.